Arginine in burn injury improves cardiac performance and prevents bacterial translocation

JURETA W. HORTON, JEAN WHITE, DAVID MAASS, AND BILLY SANDERS
Department of Surgery, The University of Texas Southwestern Medical Center, Dallas, Texas 75235-9160

Horton, Jureta W., Jean White, David Maass, and Billy Sanders. Arginine in burn injury improves cardiac performance and prevents bacterial translocation. J. Appl. Physiol. 84(2): 695–702. 1998.—This study examined the effects of arginine supplement of fluid resuscitation from burn injury on cardiac contractile performance and bacterial translocation after a third-degree burn comprising 43% of the total body surface area in adult rats. Before burn injury, rats were instrumented to measure blood pressure; after burn (or sham injury), paired groups of sham-burned and burned rats were given vehicle (saline), L-arginine, D-arginine, or N-methyl-L-arginine (300 mg/kg in 0.3 ml of saline 30 min, 6 h, and 23 h postburn) plus fluid resuscitation; sham-burned rats received drug only. Twenty-four hours after burn trauma, hemodynamics were measured; the animals were then killed and randomly assigned to Langendorff heart studies or to studies examining translocation of gut bacteria. Burn rats treated with vehicle, D-arginine, or N-methyl-L-arginine had well-defined cardiocirculatory responses that included hypotension, tachycardia, respiratory compensation for metabolic acidosis, hypocalcemia, cardiac contractile depression, and significant bacterial translocation. Compared with values measured in vehicle-treated burn rats, L-arginine given after burn improved blood pressure, prevented tachycardia, reduced serum lactate levels, improved cardiac performance, and significantly reduced bacterial translocation, confirming that L-arginine administration after burn injury provided significant cardiac and gastrointestinal protection. Circulating neutrophil counts fell after burn trauma and serum glucagon levels rose, but these changes were not altered by pharmacological intervention. Our finding of significantly higher coronary perfusate guanosine 3',5'-cyclic monophosphate concentration in L-arginine-treated burn rats suggests that the beneficial effects of L-arginine were mediated by nitric oxide production.

 cardiovasc. Res. 33: 12, 20, 30. Numerous studies have proposed that the loss of gastrointestinal mucosal integrity and bacterial translocation are initiating events in the devastating sequelae of burn trauma, contributing to multiple-organ failure and death (2, 17, 19).

The present study was designed to examine the effects of L-arginine supplementation of fluid resuscitation on postburn bacterial translocation and cardiac mechanical function. The stereospecific effects of L-arginine were examined by including animal groups treated after burn trauma with D-arginine, and the specific contribution of arginine-mediated nitric oxide (NO) production was examined by measuring guanosine 3',5'-cyclic monophosphate (cGMP) formation. In addition, the postburn cardiocirculatory effects of N-methyl-L-arginine, an agent that blocks NO production from arginine, were examined. While the protective effects of arginine administration after burn trauma are likely multifactorial, the data from the present study confirm that L-arginine-supplemented fluid resuscitation reduced the incidence of microbial translocation and significantly improved postburn cardiac contractile performance.

MATERIALS AND METHODS

Adult Sprague-Dawley rats weighing 300–350 g were purchased from Harlan Laboratories (Houston, TX) and allowed to accommodate to their surroundings for 4–5 days before study. Commercial rat chow and tap water were available before and after the burn injury. All animals were used in accordance with guidelines provided by the University of Texas Southwestern Medical School's Institutional Animal Care and Research Advisory Committee; in addition, animal handling was in compliance with guidelines from the American Physiological Society and the National Institutes of Health.

Catheter placement and burn procedure. Twenty-four hours before burn trauma, the rats were lightly anesthetized with methoxyflurane, and body weight was measured. Body hair on the abdomen, both sides, and the back was closely clipped, and the neck region was shaved and prepared with a surgical scrub (Betadine). A polyethylene catheter (PE-50 tubing) was inserted into the left carotid artery, with the tip advanced retrograde to the level of the aortic arch; in addition, a polyethylene catheter (PE-50) was placed in the right external jugular vein for administration of fluids and drugs. The catheters were filled with heparinized saline and exteriorized at the nape of the neck, and the skin was closed. After instrumentation, rats were housed in individual cages, and

http://www.jap.org 0161-7567/98 $5.00 Copyright © 1998 the American Physiological Society
body temperature was maintained throughout the experimental period with heating pads and heating lamps.

Hemodynamic, metabolic, and hematologic measurements were collected 18 h after catheter placement (preburn data); the animals were then deeply anesthetized with methoxyflurane and secured in a constructed template device as previously described by this laboratory (23). The surface of the area of the skin exposed through the device was immersed in 100°C water for 12 s on each side; by using this technique, full-thickness dermal burns comprising 43 ± 1% of the total body surface area were obtained. This burn technique produces complete destruction of the underlying neural tissue and a transient (< 45-s) increase in internal body temperature of 1–3°C. Sham-burn or control rats were subjected to identical preparation, except they are immersed in room temperature water. After immersion, the rats were immediately dried, and each animal was returned to an individual cage; the external jugular catheter was then connected to a swivel device (model 923 Holter pump, Critikon, Tampa, FL) for fluid administration during the 24-h postburn period (4 ml·kg⁻¹·%burn⁻¹, Parkland formula). In the sham-burn groups, the external jugular vein was cannulated but no fluid resuscitation was administered. Twenty-four hours after burn injury (or sham burn), hemodynamic parameters, including systemic blood pressure (model IDP23 Gould-Statham pressure transducer, Gould Instruments, Oxnard, CA, connected to a recorder, model 7D Polygraph, Grass Instruments, Quincy, MA) and heart rate (model 7P4F tachycardiograph, Grass Instruments), were measured, and a small sample of arterial blood (~ 0.40 ml) was withdrawn from the arterial catheter for measuring packed cell volume, hematocrit, arterial pH, blood gases, lactate, and serum electrolytes. Serum glucagon levels were measured by radioimmunoassay courtesy of Dr. Roger Unger (Veterans Affairs Medical Center, Dallas, TX). Body temperature was measured with a rectal temperature probe (model 44TA YSI-Tele Thermometer, Yellow Springs Instruments, Yellow Springs, OH), and respiratory rate was monitored by counting respiratory movement.

Experimental groups. As shown in Table 1, a total of 74 sham-burn rats (controls) were subdivided into four experimental groups to examine the effects of pharmacological intervention in the absence of burn trauma. In addition, 89 rats given a third-degree scald burn comprising 43% of the total body surface area were fluid resuscitated with lactated Ringer solution according to the Parkland formula (4 ml·kg⁻¹·%burn⁻¹), and this fluid-resuscitation regimen was continued for 24 h postburn. These burned rats were divided into four experimental groups to examine the effects of L-arginine, D-arginine, or N-methyl-L-arginine. In group 1 (sham-burn rats; n = 20) and group 5 (burn rats; n = 22), the animals received vehicle (0.3 ml saline); these rats received no pharmacological intervention, and only fluid resuscitation from burn injury was given, as described in Catheter placement and burn procedure. In group 2 (sham-burn rats; n = 18) and group 6 (burn rats; n = 21), rats received L-arginine hydrochloride (300 mg/kg in a total volume of 0.3 ml saline given intraperitoneally 30 min, 6 h, and 23 h after burn trauma or sham burn). In group 3 (sham-burn rats; n = 19) and group 7 (burn rats; n = 27), rats were given D-arginine (300 mg/kg in 0.3 ml saline) at the identical time periods described for groups 2 and 6. In group 4 (sham-burn rats; n = 17) and group 8 (burn rats; n = 19), N-methyl-L-arginine was administered as 100 mg/kg body wt at the time intervals and routes of administration described above. L-Arginine, D-arginine and N-methyl-L-arginine were obtained from Sigma Chemical (St. Louis, MO); each drug was dissolved in normal saline, and the pH was adjusted to 7.4, which ensured that physiologic responses observed could not be attributed to changes in arterial pH after pharmacological intervention.

Isolated coronary perfused hearts. After collection of a blood sample and measurement of hemodynamic parameters, eight to nine awake rats from each of the eight experimental groups (Table 1) were anticoagulated with heparin sodium (1,000 U, Elkins-Sinn, Cherry Hill, NJ) and decapitated with a guillotine; the heart was rapidly removed and placed in ice-cold (4°C) Krebs-Henseleit bicarbonate-buffered solution [containing (in mM) 118 NaCl, 4.7 KCl, 21 NaHCO₃, 2.5 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, and 11 glucose]. All solutions were prepared each day with demineralized, deionized water and were bubbled with 95%O₂-5% CO₂ (pH 7.4, PO₂ 550 Torr, PCO₂ 38 Torr). A 17-gauge cannula placed in the ascending aorta was connected via glass tubing to a buffer-filled reservoir for perfusion of the coronary circulation at a constant flow rate of 6 ml/min. Hearts were suspended in a temperature-controlled chamber maintained at 36 ± 0.5°C, and a constant-flow pump (model 911 Holter, Critikon) was used to maintain perfusion of the coronary artery by retrograde perfusion of the aortic stump cannula. The Krebs buffer was passed through a bubble trap and heating coil before delivery to the aorta. A pressure transducer, connected to the pressure tubing between the heart and the heating coil, was used to measure coronary perfusion pressure; coronary effluent was collected in a graduated cylinder and measured to confirm coronary flow rate. Contractile function was assessed, as previously described, by measuring intraventricular pressure with a saline-filled latex balloon attached to a polyethylene tube and threaded into the left ventricular chamber through an incision in the ventricular apex (23). Peak systolic left ventricular pressure was measured with a Statham pressure transducer (model P23ID, Gould Instruments) attached to the balloon cannula, and the maximum rate of left ventricular pressure (LVP) rise (+dP/dtₘₚₓ) and fall (−dP/dtₘₜₓ) was obtained using an electronic differentiator (model 7P20C, Grass Instruments); all parameters were recorded on an ink-writing recording system (model 7DWL8P, Grass Instruments).

A Frank-Starling relationship for all hearts was determined by plotting peak systolic LVP and ±dP/dtₘₚₓ values against incremental increases in left ventricular end-diastolic volume. The capacity of each intraventricular balloon was determined by recording the pressure-volume filling curve of the isolated balloon, and experiments were per-

<table>
<thead>
<tr>
<th>Table 1. Experimental design</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sham Burn</strong></td>
</tr>
<tr>
<td><strong>No. of rats</strong></td>
</tr>
<tr>
<td>Group no.</td>
</tr>
</tbody>
</table>

n, No. of rats. Animals within each experimental group were subdivided for either isolated heart perfusion (8–9 rats/group) or for bacterial translocation studies (10–12 rats/group).
formed on the flat area of the pressure-volume curve. Because heart rate varied after burn injury, hearts were paced at levels measured in hearts from sham-burned rats through an electrode attached to the sinoatrial node (4.2–4.6 A, 1-ms duration; Grass stimulator, Grass Instruments). Thus differences in left ventricular performance measured in hearts from burned vs. control rats could not be attributed to the differences in heart rate. In addition, ventricular performance was assessed in all hearts as coronary flow rate was increased from 3 to 12 ml/min.

Cardiac perfusate was collected after the initial stabilization period (constant coronary flow rate of 6 ml/min and an end-diastolic pressure of 10 mmHg); perfusate creatine kinase (CK), lactate dehydrogenase (LDH), and lactate were measured by using enzymatic techniques (Sigma Chemical). cGMP concentration was measured in coronary perfusate with a commercially available enzyme immunoassay (Amer-sham International). All perfusate samples for cGMP assay were snap-frozen in liquid nitrogen and stored at -80°C to prevent storage-related alterations before assay. Standards ranging from 2 to 1,510 fmol/50 μl were prepared from a stock solution, and an acetylation assay was used for all perfusate samples; cGMP was reported as fmoles per milliliter perfusate.

After rats were killed and the hearts were removed for cardiac perfusion studies, sections (100 mg) of lung and small bowel (ileum) were collected, and wet weights were measured (metric scale, model 9500, Cahn Instruments, Cerritos, CA). Tissues were placed in a drying oven (model Imperial III radiant-heat oven, Lab-Line Instruments, Melrose Park, IL), dried for 48 h, and then weighed. Water content was calculated as milliliters of water per gram dry weight of tissue.

Translocation of gut bacteria. In additional groups of sham-burned controls and burned animals, translocation of indigenous bacteria was studied. Twenty-four hours after burn injury (or sham burn for control animals), the rats were anesthetized (methoxyflurane); a 1-ml aliquot of blood was obtained anaerobically by cardiac puncture. Peripher al organs were tested for translocating bacteria, as previously described (3, 12, 19, 20); by using sterile technique, the mesenteric lymph nodes (MLN), spleen, and liver were removed, and the peritoneal cavity was cultured. The organs were homogenized in 0.15 M phosphate-buffered saline (pH 7.20), and aliquots (1 ml) of organ homogenate as well as aliquots of blood obtained from cardiac puncture were plated onto MacConkey agar and blood agar plates (Austin Biological Labs, Austin, TX). Plates were examined at 24 and 48 h after aerobic incubation at 37°C. After the MLN, liver, and spleen had been removed for culture, the cecum was removed, weighed, and homogenized in phosphate-buffered saline. Serial dilutions of the cecal homogenate were plated on MacConkey agar, and the plate was incubated and read.

Statistical analysis. All values are presented as means ± SE. Statistical comparison of group values included an analysis of variance and multiple-comparison procedure (Newman-Keuls). Relative changes in contractile performance to altered coronary flow rate were compared, as well as differences or similarities between performance-flow relationships achieved in hearts of control and burn rats. Multiple regression analysis of best-fitting curves with test evaluation was included. Probability values ≤0.05 were considered significant. The incidence of bacterial translocation was evaluated by χ²-analysis. Differences were considered significant at P < 0.05.

RESULTS

Mean arterial blood pressure, heart rate, packed cell volume, body temperature, and acid-base balance were similar in all sham groups, regardless of pharmacological intervention (Table 2). Major burn produced a well-defined cardiocirculatory response that included hypotension and tachycardia; respiratory compensation for metabolic acidosis was evident from the increased respirations per minute in all burn rats paralleled by a significant rise in arterial lactate levels regardless of pharmacological intervention. Hypocalcemia occurred after burn trauma, as indicated by a fall in serum calcium measured in the vehicle-treated burn rats from 2.56 ± 0.03 to 0.99 ± 0.01 mg/dl. These hemodynamic and metabolic responses to burn trauma occurred despite aggressive fluid resuscitation in all burned animals. L-Arginine supplementation of fluid resuscitation from burn trauma (group 6) improved mean arterial blood pressure compared with values measured in vehicle-treated burn rats (group 5), prevented burn-induced tachycardia, reduced serum lactate levels compared with values measured in vehicle-treated burn rats, but did not return serum lactate levels to those measured in sham-burn controls. In contrast, tachycardia persisted in burn rats treated with either D-arginine (group 7) or N-methyl-L-arginine (group 8); and serum lactate levels measured in these groups were similar to those measured in vehicle-
treated burns (group 5). Serum CK and LDH were significantly elevated in all burn rats irrespective of group assignment and drug administration (Table 2).

Cardiac mechanical function and biochemical markers of cardiac injury are described for all experimental groups in Table 3. There were no significant differences in LVP, $\pm dP/dt_{max}$, coronary perfusion pressure, or coronary vascular resistance among the sham groups regardless of pharmacological intervention. Hearts from the vehicle-treated burns (group 5), $\alpha$-arginine-treated burn (group 7), and N-methyl-$L$-arginine-treated burn rats (group 8) generated significantly lower peak systolic LVP and $\pm dP/dt_{max}$ values compared with values measured in sham groups. In contrast, administration of L-arginine after burn trauma (group 6) improved most indexes of cardiac contractile performance compared with those measured in vehicle-treated burn rats; but the biochemical marker of cardiac injury (CK) was elevated significantly and similarly in the coronary perfusate from all burn rats ($P < 0.05$; Table 3), confirming that functional protection was not paralleled by reduced CK leakage and reduced cell death.

Comparison of ventricular performance in sham groups given vehicle, L-arginine, $\alpha$-arginine, or N-methyl-$L$-arginine and studied as either preload or coronary arterial perfusion pressure improved cardiac contractile performance, as indicated by the ability of hearts from this burn group to produce cardiac contractile responses that were nearly identical to those of L-arginine-treated sham rats (Fig. 2B).

Figure 3 compares peak systolic LVP and $\pm dP/dt_{max}$ responses to increases in preload in vehicle-treated burn rats as well as L-arginine- and $\alpha$-arginine-treated burn groups. These studies confirm the stereospecific effects of L-arginine treatment, because $\alpha$-arginine was ineffective in preventing postburn cardiac contractile depression.

Bacterial translocation occurred 24 h after vehicle treatment, with 60% of the burn rats exhibiting bacteria in the MLN compared with 8% in the sham-burn rats (Fig. 4). Similarly, the incidence of postburn bacterial translocation to spleen (50%) and liver (50%) was significantly higher than that measured in the sham-burn group (8% in spleen and liver, respectively). L-Arginine, but not $\alpha$-arginine or N-methyl-$L$-arginine, reduced the incidence of burn-related bacterial translocation. We defined a positive culture as any growth of enteric bacteria in MLN, spleen, or liver. However, the number of colony-forming units (CFU) per gram tissue

### Table 3. Cardiac mechanical function and biochemical markers of cardiac injury measured 24 h postburn

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham-Burn Rats</th>
<th>Burn Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LVP, mmHg</td>
<td>LVP, mmHg</td>
</tr>
<tr>
<td>Group 1</td>
<td>89 ± 3</td>
<td>56 ± 4*</td>
</tr>
<tr>
<td>Group 2</td>
<td>80 ± 1</td>
<td>75 ± 2†</td>
</tr>
<tr>
<td>Group 3</td>
<td>79 ± 3</td>
<td>1,060 ± 90*</td>
</tr>
<tr>
<td>Group 4</td>
<td>80 ± 2</td>
<td>1,825 ± 125†</td>
</tr>
<tr>
<td></td>
<td>$\pm dP/dt_{max}$, mmHg/s</td>
<td>$\pm dP/dt_{max}$, mmHg/s</td>
</tr>
<tr>
<td>Group 1</td>
<td>2,199 ± 32</td>
<td>875 ± 80*</td>
</tr>
<tr>
<td>Group 2</td>
<td>1,695 ± 131</td>
<td>1,409 ± 118‡</td>
</tr>
<tr>
<td>Group 3</td>
<td>1,793 ± 111</td>
<td>1,327 ± 143</td>
</tr>
<tr>
<td>Group 4</td>
<td>1,700 ± 80</td>
<td>1,343 ± 75</td>
</tr>
</tbody>
</table>

Values are means ± SE. LVP, left ventricular pressure; $\pm dP/dt_{max}$, maximum rate of LVP rise and fall, respectively; $\Delta p/\Delta t_{max}$, rate of LVP development at a developed pressure of 40 mmHg; TPP, time to peak pressure; CPP, coronary peak pressure; CVR, coronary vascular resistance; CK, creatine kinase. * Significant difference among groups, $P < 0.05$. † Significant difference from vehicle-treated burns, $P < 0.05$.
was significantly higher in burn rats compared with the number observed in sham rats (data not shown). All blood and peritoneal cavity cultures were sterile regardless of burn injury or pharmacological intervention. The cecal levels of gram-negative enteric bacteria were similar in sham (5.1 \times 10^8 CFU), vehicle-treated burn (5.4 \times 10^8 CFU), L-arginine-treated burn (7.4 \times 10^8 CFU), d-arginine-treated burn (5.7 \times 10^8 CFU), and N-methyl-L-arginine-treated burn rats (6.1 \times 10^8 CFU). Therefore, the higher incidence of translocation in rats after burn injury could not be attributed to disruption of the normal gastrointestinal flora.

That burn trauma produced significant cardiac injury was evident from the increased level of CK in cardiac perfusates collected from hearts from burn rats (Table 3). In addition, cardiac tissue lactate levels measured in separate groups of freeze-clamped hearts confirmed postburn increases in tissue lactate in vehicle-treated burn rats (4.9 \pm 0.2 mol/g wet wt) compared with values measured in sham rats (1.78 \pm 0.19 mol/g wet wt; \(P < 0.05\)). L-arginine treatment of burn trauma decreased cardiac tissue lactate levels (2.4 \pm 0.04 mol/g wet wt) compared with values measured in vehicle-treated burn rats (\(P < 0.05\)).

Fig. 2. Comparison of ventricular responses to increases in left ventricular volume in burn rats (Burns) given vehicle, L-arginine, d-arginine, or N-methyl-L-arginine. Values are means \(\pm\) SE. \(t\)-test. *Significant difference among groups, \(P < 0.05\).}

\[\text{L-Arginine administration during the postburn period increased cGMP levels above those measured in perfusates from hearts from sham-burn rats, and cGMP levels were significantly different from those measured in vehicle-treated burn, d-arginine-treated burn, or N-methyl-L-arginine-treated burn rats (\(P < 0.05\)).} \]

Circulating neutrophil counts tended to fall after burn trauma (burn rats, 6,600 \pm 630/mm³; sham-burn rats, 7,243 \pm 1,230/mm³), but this change was not significant nor was it altered by any pharmacological intervention. We also considered that L-arginine administration may have provided cardioprotection by enhancing pancreatic release of glucagon, a powerful cardiac inotrope. Serum concentration of glucagon rose after vehicle treatment in burn rats (789 \pm 59\, pg/ml) and was significantly elevated compared with values measured in sham rats (308 \pm 46\, pg/ml; \(P < 0.05\)). However, glucagon levels were similar in all burn groups regardless of pharmacological intervention (L-arginine, 578 \pm 59\, pg/ml; d-arginine, 658 \pm 81\, pg/ml; and N-methyl-L-arginine 747 \pm 198\, pg/ml), likely indicative of the overall stress hormone response to major trauma. Burn trauma also increased lung and intestinal water content (4.52 \pm 0.74 and 6.75 \pm 1.0\, ml H₂O/g dry wt, respectively) compared with values measured in sham rats (lung 3.95 \pm 0.09 ml H₂O/g dry wt; intestine 2.82 \pm 0.22 ml H₂O/g dry wt; \(P < 0.05\)). All regimens of pharmacological intervention decreased both lung and intestinal water content (L-arginine, 3.98 \pm 0.34 and 3.22 \pm 0.12; d-arginine, 3.63 \pm 0.08 and 3.08 \pm 0.06; N-methyl-L-arginine 3.76 \pm 0.08 and 3.18 \pm 0.10 ml H₂O/g dry wt, respectively).

Fig. 3. Cardiac contractile function after burn trauma; L-arginine but not d-arginine enhanced LVP and maximum rate of LVP rise and fall (\(\pm\)dP/dt\text{max}) responses to increases in left ventricular volume. Values are means \(\pm\) SE. *Significant difference among groups, \(P < 0.05\) (analysis of variance and Student-Newman-Keuls test).
DISCUSSION

In this present study, administration of L-arginine during fluid resuscitation from a major burn injury in rats provided significant cardioprotection and reduced the incidence of bacterial translocation from the gastrointestinal tract. The cardioprotective benefits of L-arginine were stereospecific, because D-arginine failed to either improve cardiac contractile function or alter the incidence of postburn bacterial translocation. All burned groups were given comparable percentage total body surface area burns and received identical regimens of lactated Ringer fluid resuscitation. In addition, all animals were randomized to receive one of the pharmacological interventions, and there were no significant differences in the times required to harvest the heart for in vitro perfusion. Thus the differences in cardiac contractile responses observed 24 h after burn injury could not be attributed to differences in animal handling, volumes of fluid administered throughout the postburn period, or technical differences in isolation and handling of perfused organs.

Numerous studies have demonstrated that a major burn injury alters cardiovascular performance, and both clinical and experimental studies have shown that the postburn decrease in cardiac output does not parallel the decrease in plasma volume (9, 22, 24). Furthermore, volume resuscitation to replace or exceed intravascular fluid loss does not always restore left ventricular stroke volume (9, 22, 24). In addition, experimental studies in several animal models, including rats, guinea pigs, rabbits, and sheep, have shown that burn trauma produces cardiac contractile deficits that are intrinsic to the cardiac myocyte and are not Frank-Starling responses to the cardiac myocyte and are not

In addition to the cardiocirculatory defects, which have been shown to occur after several types of trauma, including burn injury, disruption of the intestinal mucosal barrier and emigration of bacteria have been recognized as a hallmark of stress-related injury (3, 12, 17, 19, 20). The passage of microorganisms and endotoxin through the gastrointestinal mucosal cells has been identified as a major factor in the development of multiple-organ failure and has been implicated in the pathogenesis of endotoxemia, the septic syndrome, hemorrhagic shock, and, more recently, burn trauma (6, 8, 11, 13, 31). Previous studies have suggested that postburn splanchnic hypoperfusion, a phenomenon that occurs within the first 90 min after a major burn injury, is a major factor in the devastating consequences of bacterial translocation, cytokine production, and downstream organ injury (25, 28, 36).

In the present study, bacterial translocation occurred after burn trauma, despite aggressive fluid resuscitation, confirming previous reports of postburn increases in gut permeability to enteric bacteria (2, 11, 12, 17, 19, 20). Our finding that L-arginine plus fluid resuscitation reduced the incidence of bacterial translocation to MLN, liver, and spleen contrasts with a report by Gianotti and colleagues (16), who showed that the magnitude of translocation to the liver and spleen was not altered by dietary arginine; however, in that previous study, an arginine-supplemented diet reduced microbial translocation to the MLN and enhanced the ability to kill translocated organisms. The beneficial effects of arginine on intestinal barrier function in our study may have been related to the fact that arginine is a precursor of several polyamines that play an essential role in cell growth and differentiation (29). In this regard, several studies showed that oral or intravenous arginine stimulated the release of several hormones that exert trophic effects on the intestinal mucosa (37), and these hormones may in turn prevent the stress-related bacterial translocation. Alternatively, arginine may have upregulated NO production by the mesenteric vascular endothelium, preventing burn-induced mesenteric hypoperfusion and contributing to the main-
tenance of intestinal integrity (4). Alternatively, the reduced incidence of bacterial translocation in the L-arginine-treated burn rats may have been related to the bactericidal activity of NO and the inactivating and/or scavenging of oxygen-derived free radicals within the mesenteric circulation (14). Although the data from the present study clearly document the protective effects of L-arginine on intestinal barrier integrity after burn injury, the precise mechanisms remain unclear.

We also considered that the protective effects of L-arginine in this study were related to increased NO production by the coronary endothelium, preserving endothelial integrity and enhancing perfusion of peripheral organs (26, 34). We have previously shown that L-arginine improved cardiac contractile performance in a model of transient intestinal ischemia and reperfusion by increasing coronary blood flow, and, in that study, L-arginine and nitroglycerine were equally cardioprotective (33). To address the issue that L-arginine may directly alter coronary endothelial NO production, we measured cGMP concentration in the coronary effluent from isolated perfused hearts. The data from the present study support our previous finding that burn trauma decreased coronary cGMP levels (14); in addition, cGMP levels were significantly higher in coronary effluents from L-arginine-treated burn rats, suggesting enhanced cardiac NO production. It is likely that providing L-arginine as a substrate for NO synthesis by the coronary endothelium throughout the 24-h postburn period prevented coronary hypoperfusion, which we have previously shown to occur in experimental burns (unpublished data).

Numerous studies have debated the role of NO in several models of trauma, and several investigators have demonstrated beneficial effects of NO, whereas others have suggested that NO, like other free radicals, contributes to peroxidative damage to cell membranes. In light of this continuing controversy, we also considered that the protective effects of L-arginine on postburn cardiac performance and intestinal mucosal integrity were independent of L-arginine as a substrate for NO synthase. Al-Rajab et al. (5) demonstrated that L-arginine administration increased pancreatic release of the potent cardiac inotrope glucagon. Therefore, in this present study, we hypothesized that L-arginine administration after burn trauma produced hyperglucagonemia, increasing cardiac contractility and cardiac output and subsequently improving splanchnic perfusion. However, in our study, serum glucagon levels were elevated to a similar extent in all burn groups regardless of pharmacological intervention, likely reflecting the overall hormonal response to stress injury. Therefore, we concluded that an arginine-mediated increase in glucagon release was not the mechanism of cardioprotection.

Another mechanism by which L-arginine may have afforded organ protection after burn trauma is decreased neutrophil aggregation and activation, down-regulation of the overall inflammatory response to burn injury, and a decrease in cytokines and cytotoxic mediator production (2). Saito and colleagues (35) showed that arginine-supplemented diets improved cell-mediated immunity, enhanced wound healing, and improved survival in experimental burn trauma. Clinical studies have shown that dietary arginine enhanced natural killer cell activity and survival in models of infection, burn, and transfusion-induced immunosuppression (17), decreased the incidence of infectious complications, and improved overall outcome in surgical patients (10, 13, 35). Our previous studies have shown increased tumor necrosis factor-α production by cardiac myocytes, endothelial injury and dysfunction, and neutrophil emigration into cardiac tissue after burn injury (24), confirming that our model of thermal trauma produces an inflammatory sequelae typical of major injury (14, 34). Although the present study did not specifically examine neutrophil adherence and activation, we did show that circulating neutrophil counts tended to fall over the first 24 h after burn injury, suggesting sequestration of this cell population in the microvasculature and/or emigration into tissue. Our previous studies showed that inhibition or depletion of neutrophils reduced postburn myocardial injury and dysfunction (24), suggesting that one mechanism of L-arginine-mediated protection is NO-mediated inhibition of neutrophil adherence and activation.

Another mechanism by which arginine administration may achieve organ protection is the insulin response to arginine infusion. It is well recognized that arginine stimulates insulin secretion, which, in turn, may promote vasodilatation. However, previous studies have demonstrated that both L-arginine and D-arginine increased serum insulin levels to a similar extent in rats (18). Because cardiac and intestinal mucosal protection in our study were achieved only with L-arginine administration, it is unlikely that the protective effects were related to arginine-mediated increases in insulin secretion.

Although our study examined cardiac contractile function and bacterial translocation in a rat model of thermal injury and extrapolation of the data to the human burn subject must be approached with caution, our data provide unequivocal evidence that L-arginine administration after burn injury provided significant cardiac and gastrointestinal protection. However, our finding that CK was elevated in coronary effluent from L-arginine-treated burned animals suggests that L-arginine was not protective against cardiac cellular damage. Although it is attractive to hypothesize that the beneficial effects of L-arginine were related to upregulation of NO production, maintenance of vasmotor tone, and a flow-related phenomenon, the effects of L-arginine on the inflammatory response to burn trauma cannot be ruled out. Our data suggest that therapeutic interventions which increase production of NO from L-arginine after burn trauma provided gastrointestinal protection and improved cardiac mechanical performance.
ARGinine IN BURN TRAUMA

Address for reprint requests: J. W. Horton, Dept. of Surgery, The Univ. of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9160.

Received 14 July 1997; accepted in final form 25 September 1997.

REFERENCES


